World Journal of Medical Sciences 9 (4): 248-253, 2013
ISSN 1817-3055

DOI: 10.5829/idosi.wjms.2013.9.4.81122

Prevalence of Genital Mycoplasmas Inthe Vaginal Tracts of Adolescents in Nnewi, South-Eastern, Nigeria

[1]Chukwuka Chiamaka P., [1]Agbakoba Nneka R.,
[1]Emele Felix E., [2]Oguejiofor Chariot, [1]Akujobi Comfort N.,
[3]Ezeagwuna Dorothy A. and [3]Onwunzo Maria C.

[1]Department of Medical Microbiology and Parasitology, Faculty of Medicine,
College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria
[2]Department of Obstetrics and Gynaecology,
Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria
[3]Department of Medical Microbiology, Nnamdi Azikiwe University Teaching Hospital,
Nnewi, Anambra State, Nigeria

Abstract: This study aims to screen adolescent girls in Nnewi, South-Eastern Nigeria for the presence of potential pathogens like genital mycoplasmas and also to know the associated risk factors predisposing them to these organisms. A cross-sectional study involving the use of high vaginal swabs (HVS) from 100 adolescent girls aged between 13 and 18 years was done. Molecular method was used to analyse the swabs using the polymerase chain reaction technique. Questionnaires were also used to obtain the bio data and evaluate the risk factors to these vaginal organisms. A prevalence rate was was 20% out of which 4% represented concomitant colonization by 2 or more different species. A breakdown of the organisms showed that the Ureaplasma species-*Ureaplasma urealyticum* and *Ureaplasma parvum* had 6% and 4% colonization rates respectively while the Mycoplasma species-*Mycoplasma hominis* and *Mycoplasma genitalium* had 4% and 6% rates respectively. Poor personal hygiene and sharing of personal effects were found to be risk factors in predisposing subjects to *M. hominis* acquisition. The high prevalence of these organisms among asymptomatic adolescents suggests strongly that they are not always associated with symptoms thus supporting the need for screening among this population.

Key words: Genital mycoplasmas · Adolescent girls · Risk factors

INTRODUCTION

Human genital mycoplasmas are species of Mycoplasmas and Ureaplasmas isolated from the urogenital tracts of humans. Most often they exist asymptomatically in the genital tracts, however; they have been associated with bacterial vaginosis along with other pathogens like *Gardnerella vaginalis*,

Mobiluncus species and vaginal anaerobes [1]. They have also been implicated in pelvic inflammatory disease (PID), infertility and various other genital pathologies [2].

The role of genital mycoplasma as a disease-causing agent has been reported in many parts of the world [3, 4]. Although very limited studies have been done in Nigeria [5, 6], none has been carried out on adolescents. Despite the reports on these organisms, they are still not being investigated routinely mainly due to their fastidious nature and the technically challenging culture methods needed to link the organism to clinical conditions [2]. The availability of molecular methods has substantially altered the ability to derive information about the pathogenic potentials of these groups of bacteria in affecting the reproductive health of adult females [7].

Adolescents are physiologically more vulnerable to infection than older women because changes in the reproductive tract during puberty make their vagina and

Corresponding Author: Agbakoba Nneka, Department of Medical Microbiology and Parasitology, Faculty of Medicine, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

cervix less resistant to infection [8]. It is known that it is the many activities of genital mycoplasmas and their prolonged asymptomatic presence in the reproductive tract that causes infertility [9]. Since it has been shown that interventions are not usually successful once complications have set in, it becomes important to generate and provide research information for policy makers, health-care providers, parents and the general populace, so that appropriate attention will be given to the youth at an early age.

The adolescent years are highly influential in shaping their adulthood, particularly with regards to hygiene and other lifestyle habits. Intervention aimed at this population may help reduce the risk posed by potential genital tract pathogens. Although screening of youths for harmful genital pathogens have been recommended by the world health organization [10], nothing of such is being carried out on genital mycoplasmas, probably because little information is available here in Nigeria among young adolescent female population. The sexual and reproductive health indices of the adolescents are very poor as depicted in the 2008 Nigerian demographic and Health survey [11]. This research is therefore aimed at bridging this gap.

In Nigeria, as well as in other developing countries, little is known about the prevalence of genital mycoplasmas among adolescent females, therefore the study the study aimed to spot on the to spot on the prevalence among adolescent high school female students in Nnewi, South-Eastern, Nigeria.

MATERIALS AND METHODS

Subjects: The study population comprise 100 female high school students aged between 13 to 18 years and attending school in Nnewi, South-eastern, Nigeria. Written informed consent was obtained from their parents/guardians while oral inform consent was obtained from the girls at collection point.

Samples: One high vaginal swab was taken from each of the students by a clinician using sterile disposable cotton swab stick. The swabs were inserted into 0.5ml of phosphate buffered saline and stored at 4°C prior to PCR assay. Immediately after sample collection, each subject was given a questionnaire to fill. This contains the bio data and various risks factors.

Exclusion Criteria: Menstruating girls and those that have taken antibiotics or antifungal a month before specimen collection were excluded.

DNA Extraction: The DNA was extracted from the samples in a stepwise procedure using the Trizol reagents as recommended by the manufacturer (Invitrogen, UK). This procedure includes the phase separation stage, the DNA precipitation, DNA wash and re-dissolving DNA.

Oligonucleotide Primers: Four (4) primer pairs based on previously published sequences [12-14] were used. They include;

UMA-51-F and UMA-427 R for *Ureaplasma parvum* (Biovar 1),
UMS 125-F and UMA-226R for *U. urealyticum* (Biovar 2),
My-insF and Mgen-P3-AMR for *Mycoplasma genitalium* and
My-ins-F and Mhom-P-10-AM-R for *Mycoplasma hominis*;

Preparation of Master Mix: The master mix used was 2X Qiagen multiplex PCR master mix. This PCR kit provides the multiplex PCR master mix containing hot-start Taq DNA polymerase and a unique PCR-buffer containing factor MP. Together with optimized salt concentrations, the factor MP stabilizes specifically bound primers and enables efficient extension of all primers in the reaction without optimization.

PCR Mix: Each sample is tested against all four mixes and the amplification reaction mixture for one sample includes 7µl of primer mix, 10µl of PCR master mix and 3µl of genomic DNA to give a final volume of 20µl.

PCR: The 20µl PCR mix of each subject were put in 1.5ml micro centrifuge tubes and transferred to a thermal cycler (2720 Applied Biosystem). The thermal profiles include initial denaturation step at 95°C for 30seconds, annealing at 62°C for 45 seconds and extension at 72°C for 45 seconds. This was done for 35 cycles and followed by a final extension at 72°C for 7 minutes. This is the PCR product and was kept at a holding temperature of 20°C until ready to use. The PCR conditions were as previously described [12, 7].

Two (2µl) of the PCR products of each of the samples were analysed by electrophoresis which was ran at 100V for 30minutes. Expected bands for positivity were 423bp *for U. urealyticum*, 427bp for *U. parvum*; 520bp for *M. genitalium* and 326bp for *M. hominis*.

Statistical Analysis: Number/percentages were used to describe the data obtained and calculations between variables were explored with the chi square. $P < 0.05$ was considered significant.

RESULTS

An overall colonization rate of 20% was obtained from the study population out of which 4% represents concomitant colonization among the organisms while individual detection rates were as follows; *Mycoplasma genitalium* 6 (6%), *Mycoplasma hominis* 3 (3%), *Ureaplasma urealyticum* 2 (2%) and *Ureaplasma parvum* 1 (1%). *Mycoplasma genitalium* was the only species not co-existing with any of the three (3) other species. *Ureaplasma urealyticum* co-existed with both *Ureaplasma parvum* and *Mycoplasma hominis* (Table 1).

Distribution of genital mycoplasmas among the 2 age groups shows that *M. genitalium* was associated more among age group 13-15 years ($p < 0.05$) (Table 2).

Table 3 showed the effect of sexual habits of the adolescents on the presence of these organisms. Only 14% of the population reported to have sexual experience. Each species of mycoplasma was found to colonise only one (7.1%) of these sexually experienced girls. Though the percentage positive for genital mycoplasma among the sexually experienced adolescents is higher (7.1%) than that among the inexperienced (3.8% and 5.8%), this result is not statistically significant ($p > 0.005$).

In Table 4 the sharing of personal effects like towels, sponges and panties among siblings or friends seems to be a factor predisposing the adolescents to *Ureaplasma* infection ($P < 0.05$) while type of toilet in use (pit latrine or water cistern) and type of studentship (Day student or Boarder) seem to have no influence on colonization rate.

Table 1: Concomitant colonization of Genital mycoplasmas among the positive samples

Genital Mycoplasma species	U1 (No/%)	U2 (No/%)	Mh (No/%)	Mg (No/%)	Total (No/%)
Ureaplasmaurealyticum(U1)	2 (2.0%)	3 (3.0%)	1 (1.0%)	0 (0.0%)	6 (6.0%)
Ureaplasmaparvum(U2)	3 (3.0%)	1 (1.0%)	0 (0.0%)	0 (0.0%)	4 (4.0%)
Mycoplasma hominis(Mh)	1 (1.0%)	0 (0.0%)	3 (3.0%)	0 (0.0%)	4 (4.0%)
Mycoplasma genitalium (Mg)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (6.0%)	6 (6.0%)
TOTAL	6 (6.0%)	4 (4.0%)	4 (4.0%)	6 (6.0%)	20 (20.0%)

Table 2: Distribution of Genital mycoplasmas among the subjects according to age

	Genital mycoplasmas (No./ %)			
Age group (years)	*U. urealyticum*	*U. parvum*	*M. hominis*	*M. genitalium*
13-15	2 (5.3%)	1 (2.6%)	2 (5.3%)	5 (13.2%)
16-18	4 (6.5%)	3 (4.8%)	2 (3.2%)	1 (1.6%)
TOTAL	6 (6.0%)	4 (4.0%)	4 (4.0%)	6 (6.0%)
X^2	0.059	0.299	0.255	5.568
p-value	0.808	0.585	0.614	0.018*

* = significant result

Table 3: Effect of sexual habits on isolation of genital mycoplasmas

	Sexual Experience			
Genital mycoplasmas	Yes	No	X^2	P-value
U. urealyticum (U1)	Pos	1 (7.1%)	5 (5.8%)	
Neg	13 (92.9%)	81 (94.2%)		
Total	14 (14.0%)	86 (86%)	0.038	0.846
U. parvum	(U2)	Pos	1 (7.1%)	3(3.5%)
Neg	13 (92.9%)	83 (96.5%)		
Total	14 (14.0%)	86 (86.0%)	0.419	0.518
M. hominis (Mh)	Pos	1 (7.1%)	3(3.5%)	
Neg	13 (92.9%)	83 (96.5%)		
Total	14 (14.0%)	86 (86.0%)	0.419	0.518
M. genitalium	(Mg)	Pos	1 (7.1%)	5 (5.8%)
Neg	13 (92.9%)	81 (94.2%)		
Total	14 (14.0%)	86 (86%)	0.038	0.846

Table 4: Effect of other risk factors on colonization with genital mycoplasmas

		Genital mycoplasamas (No./%)			
Risk Factor	Status	*U. urealyticum*	*U. parvum*	*M. hominis*	*M. genitalium*
Personal hygiene	Poor	1(4.3%)	1(4.3%)	0 (0.0%)	0 (0.0%)
	Pass	0 (0.0%)	0 (0.0%)	1 (100%)	0 (0.0%)
	Good	3 (10.3%)	2(6,9%)	1 (3.4%)	1 (3.4%)
	Excellent	2(4,4%)	1(2.1%)	2 (4.3%)	5 (10.6%)
	Total	6(6%)	4(4%)	4 (4.0%)	6 (6.0%)
	X2	1.399	1.112	24.989	3.660
	P value	0.706	0.774	0.000	0.301
Sharing of personal effects	Yes	3 (25.0%)	2 (16.7%)	0 (0.0%)	0 (0.0%)
	No	3 (3.4%)	2 (2.3%)	4 (4.5%)	6 (6.8%)
	Total	6 (6.0%)	4 (4.0%)	4 (4.0 %)	6 (6.8%)
	X2	8.728	5.698	0.568	0.870
	P value	0.003*	0.017*	0.451	0.351
Toilet type	Water cistern	5 (7.1%)	3 (4.3%)	3 (4.3%)	4 (5.7%)
	Pit	1 (3.3%)	1 (3.3%)	1 (3.3%)	2 (6.7%)
	Total	6 (6.0%)	4 (4.0%)	4 (4.0%)	6 (6.0%)
	X2	0.540	0.050	0.050	0.034
	P value	0.462	0.824	0.824	0.854
Student type	Student type	No. examined	No. % positive		
		65			
	D/ student	35	12(18.5%)		
	Boarder	100	4 (11.4%)		
	Total		16(16.0%)		
	X2		0.837		
	P value		0.36		

*= significant results

Key: D/student= Day student.

DISCUSSION

An overall prevalence rate of 20% genital mycoplasmas was obtained from the adolescent girls studied. Not much report was obtained on genital mycoplasmas from adolescent girls elsewhere but Manhart *et al.* [15] who worked on *Mycoplasma genitalium* from young adults reported 0.8% prevalence rate. This is much lower than the 6% prevalence rate observed from this study on *M. genitalium*. However, higher prevalence rates have been reported from adult females; 35.7% from adult females in Ibadan, Nigeria [7], 32.5% from Northern Nigeria Jumbo *et al.* [16]; 38.0% from women attending sexually transmitted in Paris [3] and 48.0% from Turkey [17]. The lower prevalence rate obtained in this study could be as a result of the population used. They young girls probably were less exposed than the adult females.

Age distribution did not show any difference in this study except for *M. genitalium* which showed a predilection for those between 13-15 years ($P<0.05$). In agreement with our findings, Elias *et al.* [4] also found no statistical significant correlation between the age of the patient and incidence of mycoplasma. In this study, the prevalence of genital mycoplasma among the age group 13-15years was 26.4% while among 16-18 years was 16.1%. Tibaldi *et al*. [18] also reported a significantly higher risk of Ureaplasma colonization among younger women than older ones. Age therefore, is an independent risk factor.

A breakdown of the result into the two major student groups studied (Day students and Boarders) showed that a higher prevalence rate of genital mycoplasmas among the Day students (18.5%) than the Boarders (11.4%). Though not statistically significant, this result probably implies that the boarders are more aware of their hygienic routine than the Day students.

From this study, genital mycoplasmas appear not to be only sexually transmitted, as only 3.0% out of the 16.0% positivity rate were detected from the sexually experienced adolescents. According to Manhart *et al*. [15], *M. genitalium* infection was strongly associated with having engaged in vaginal intercourse. This organism has been presumed to be sexually transmitted, a conclusion made on the basis of studies on sexually active individuals [19]. *Ureaplasma urealyticum* has also been

associated with high risk behaviour [18] although in an earlier report the association of *U. urealyticum* with sexual activity are rather contradictory [20]. In contrast with all these studies, this sample population included both sexually experienced and non-experienced adolescents girls and this allowed this study to show the significant association of absence of sexual activity with genital mycoplasma detection; thereby strengthening the case for other means of transmission of these organisms. Other investigators who worked on bacterial vaginosis in sexually experienced and inexperienced young women share this opinion [21].

Risk factors for genital mycoplasma colonization among the adolescents from this study showed increased risks to be significantly ($P<0.05$) associated with sharing of personal effects and poor personal hygiene, whereas being a day or boarding student, toilet type, sexual experience had little or no significant effect on the risk for genital mycoplasma among this category of individuals. This could imply that improving the hygiene status of adolescents may reduce the colonization rate. Manhart *et al*. [15], associated increased risk for *M. genitalium* with ever having lived with a sexual partner, being black and absence of condom.

Detection of genital mycoplasma in the lower genital tract has been associated with inflammation in the upper genital tract in form of histological diagnosed endometritis, clinically diagnosed pelvic inflammatory disease (PID) [22, 23] and laparoscopically diagnosed salpingitis [24]. Although the population studied are asymptomatic, such are important because asymptomatic infections represent the carrier-state which serves as a reservoir for maintaining transmission within a population.

The findings about one-quarter of early preterm infants is already infected with genital mycoplasma at birth coupled with the fact that these new-borns had a higher incidence of neonatal systemic inflammatory response syndrome (SIRS), higher incidence of broncho-pulmonary dysplasia (BPD), higher serum concentration on interleukin (IL)-6 and more evidence of placental inflammation than those with negative cultures [25,26], goes a long way in picturing the complications and consequences associated with neglected or undetected genital mycoplasma in the reproductive tract of females. The initial uncertainties of whether genital mycoplasmas can cause serious consequences are disappearing in the light of the many accumulating evidences.

In conclusion, genital mycoplasmas are not a rare cause of symptomatic and asymptomatic female genital tract infection as have been the thinking. The high prevalence rate among the asymptomatic adolescents in this study suggest strongly that these organisms are not always associated with symptoms and this needs for screening exercise among these age group.

REFERENCES

1. Schembach, D., 1993. A history and review of bacterial vaginosis. Ann. J. Obstet. Gynecol., 169: 441-445.
2. Bryan, L. and J. Hwang, 2010. Mycoplasma, Ureaplasma and adverse pregnancy outcomes: A fresh look. Infectious diseases in Obstetrics and gynecology. Article ID:521921, pp: 5-6.
3. Casin, I., D. Vexian-Robert, P. Dela-Salmoniere, A. Eche, B. Gandry and M. Janier, 2002. High prevalence of Mycoplasma genitalium in the lower genital tract of women attending a sexually transmitted disease clinc in Paris, France. Sex. Transm. Dis., 29(6): 353-359.
4. Elias, M., J. Grzesko, R. Siejkowksi, J. Nowicka, B. Maczynska, M. Goluda and St. Gabrys, 2005. The presence of Mycoplasma hominis and Ureaplasma urealyticum in the cervical canal of the uterus. Ginecologii Poland, 76: 28-32.
5. Agbakoba, N.R., A.I. Adetosoye and I.F. Adewole, 2007. Presence of mycoplasma and ureaplasma species in the vagina of women of reproductive age. West Africa Journal of Medicine, 26(1): 28-31.
6. Jombo, G.T. and M.N. Enenebeaku, 2010. Genital mycoplasma infections among women in an urban community of northern Nigeria: do we need to search for them? Niger. J. Med., 17: 310-316.
7. Agbakoba, N.R., A.I. Adetosoye, O.A. Adesina and I.F. Adewole, 2008. Polymerase chain reaction assay of ureaplasma strains isolated from high vaginal swabs of women in Ibadan, Nigeria. African J. Med. Sci., 37: 249-254.
8. Biddle, A., L. Hessburg, S. Singh, A. Bankola and L. Darubi, 2007. Protecting the next generation in sub-Saharan African. Alan Guttmacher Int. Reports.
9. Baseman, J.B. and J.G. Tully, 1997. Mycoplasma: sophisticated, reemerging and burdened by their notoriety. Emerging infectious disease, 3(1): 21-32.
10. World Health Organization, 2006. Prevention and control of sexually transmitted infections: draft global strategy. Fifty-ninth World Health Assembly.

11. World health Organization, 2008. 10 facts on adolescents' health. WHO. Geneva.
12. Teng, L., X. Zheng, J.I. Glass, H.I. Watson, J. Tsai and G.H. Cassel, 1994. Ureaplasma urealyticumBiovar specificity and diversity are encoded in multiple-banded antigen gene. J. Clinc. Microbiol., 32(6): 1464-1469.
13. Yoshida, T., S. Maeda, T. Deguichi, T. Miyazawa and H. Ishiko, 2003. Rapid detection of Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma parvum and Ureaplasma urealyticum organisms in genitourinary sample by PCR-microtitre plate hybridzation assay.J. Clinic microbial., 41: 1850-1855.
14. Payne, M.S., K.C. Goss, G.J. Conneth, J. Kollamparambil, J.P. Legs, R. Thwantes and M. Ashton, 2010. Molecular microbiological characterization of preterm neonates at risk of brochopulmonary dysplasia. Pediatric research, 67(4): 412-418.
15. Manhart, L.E., K.K. Holmes, J.P. Hughes, L.S. Houston and P.A. Totten, 2007. Mycoplasma genitalium among young adults in the United States: An emerging sexually transmitted infection. America journal of public health, 97(6): 1118-1125.
16. Jombo, G.T.A., M.N.O. Enenebeaku, E.J. Peters, H.I. Itams and E.M. Mbaawuga, 2009. Symptomatic genital mycoplasma infections among antenatal women in an Urban Community of Northern Nigeria and the need to possibly widen the scope of present laboratory investigations.The internet journal of infectious diseases, 7(1).
17. Kiche, D., M.M. Basar, S. Kaygysuz, E. Yilmazi, H. Basar and E. Batislam, 2004. Prevalence and treatment of Chlamydia trachomatis, Ureaplasma urealyticum and Mycoplasma hominis in patients with non-gonococcal urethritis. Jpn J. Infect. Dis., 57: 17-20.
18. Tibaldi, C., N. Cappello, M.A. Latino, G. Masnelli, S. Marini and C. Benedetto, 2009. Vaginal and endocervical microorganisms in asymptomatic and symptomatic non-pregnant females: risk factors and rate of occurrence. Clinical microbiology and infection, 15(7): 670-679.
19. Kaene, F.E., B.J. Thomas, C.B. Gilroy, A. Renton and D. Taylor-Robinson, 2000. The association of Chlamydia trachomatis and Mycoplasma genitalium with non-gonococcal urethritis: observation on Heterosexual men and their females partners. Int. J. STD AIDS., 11: 435-439.
20. Schlicht, M.J., S.D. Lovrich and J.S. Sartin, 2004. High prevalence of genital mycoplasma among sexually active young adults with urethritis or cervicitis symptoms.In La Crosse, Wisconsin. J. Clin. Microbiol., 42: 4636-4640.
21. Yen, S., M. Shaffer, J. Monicada and C.J. Campbell, 2003. Bacterial vaginosis in sexually experienced and non-sexually experienced young women entering the military. Obstetrics and gynecol., 102(5): 927-933.
22. Ballard, R.C., H.G. Fehler, Y. Htun, F. Radebe, J.S. Jensen and D. Taylor-Robinson, 2002. Co-existence of urethritis with genital ulcer disease in South-Africa; Influence on provision of syndromic management. Sex transm. Infect., 78: 274-277.
23. Smith, K., K.G. Harrington, M.K.O.H. Winggood, E.W. Hook and R.J. Diclemente, 2001. Self-obtained vaginal swabs for diagnosis of treatable sexually-transmitted diseases in adolescents girls. Arch Pediatr.Adolesc. Med., 155: 676-679.
24. Cohen, C.R., N.R. Mugo and S.G. Astete, 2005. Detection of Mycoplasma genitalium in women with laparoscopically diagnosed acute salpingitis. Sex transm. Infection, 81: 463-466.
25. Romero, R. and T. Garite, 2008. Twenty percent of very preterm neonates (23 to 32 wks) are born with bacteremia caused by genital mycoplasmas. America J. Obstet and Gynecol., 198(1): 1-3.
26. Goldenberg, R.L., W.M. Andrews, R.A. Goepfert, O.F. Petersen, S.P. Cliver, W.A. Carlo and J.C. Hauth, 2008. The Alabama preterm birth study; umbilical cord blood Ureaplasma urealyticum and Mycoplasma hominis cultures in very preterm newborns. American J. Obstet. and Gynecol., 198(1): e1-43.e5.